Biologically active scaffolds: Synthesis, characterization and studies of oxino bis-pyrazoles by environmental friendly method

Noreen Aslam[1,2,] Jonathan M. White[3], Abdul Ghafoor[2,4,] Ajmal Shafique[2], Faizul Hassan Nasim[1,2,] Bakht Jahan[2], M Ashraf[2], Mussarat Jabeen[1,2,] Ansa Madiah Zafar[1,2,] Shazia Noreen[2,5,] Naveed Sajid[2,6] and Misbahul Ain Khan[2]

[1]Department of Chemistry, Govt. Sadiq College Women University Bahawalpur, Pakistan
[2]Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[3]Bio-21-Institute, University of Melbourne, Melbourne, Australia
[4]University of Sahiwal, Sahiwal, Pakistan
[5]CB Girls Public High School & College Bahawalpur, Pakistan
[6]Punjab College, Jalapur Pirwala, Pakistan

Abstract: In the present communication, synthesis of bis-pyrazolones containing aryl motifs (4-14) and their α-glucosidase inhibitory activity, hemolytic and antihemolytic activities were reported. The newly synthesized compounds were characterized by analytical techniques such ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry and compound No 4 additionally by X-ray crystallography. Compounds 4, 12, 14 were obtained in more than 85% yield. In comparison to typical *acarbose* (IC_{50} = 37.38±0.12μM), all synthesized compounds showed potent activity with IC_{50} values between 31.26±0.11 to 396.25±0.18μM. The most potent compounds 6, 8 and 11 showed IC_{50} values within the range of 31.26±0.11 to 37.48±0.12μM. Compounds 7, 10, 12 and 13 showed IC_{50} values within the range of 65.23±0.12 to 154.87±0.16μM, while compounds 4, 5 and 9 showed moderate inhibition with IC_{50} values 286.56±0.16 to 396.25±0.18μM. Structure-activity relationship (SAR) studies, suggests that electron withdrawing groups played a crucial role in enhancing α-glucosidase inhibitory effects of title compounds. In addition, results of the hemolytic and antihemolytic activity studies indicated that compound 13 possessed moderate levels of hemolytic and highest anti-hemolytic activity while 8 showed low anti- hemolytic and high hemolytic activity.

Keywords: Bis-pyrazolones, Glycine, α-glucosidase activity, Haemolytic activity, Anti-haemolytic activity, X-ray crystallography.

INTRODUCTION

Diabetes mellitus, being a serious prolonged disease is magnified globally due to genetic factor (Stumvoll *et al*., 2005) as well as by lifestyles such as obesity and aging (Kim *et al*., 2010). The α-glucosidase, an exo-type carbohydrase (Kim *et al*., 2010) located at intestinal brush-border cells (Standl and Schnell, 2012) and cleaves the α-glycosidic linkage of mainly disaccharides and oligosaccharides to increase blood glucose level (Tiengburanatam *et al*., 2010). Although, several drugs to cure type II diabetes are in use, but injuriousness to liver and heart (Kim *et al*., 2010). Thus, the comparatively new therapeutic strategy of using α-glucosidase inhibitors is gaining interest due to decreasing blood glucose levels as well as a remedy of viral infections, cancer, hepatitis (Tiengburanatam *et al*., 2010) hypertension and obesity (Standl and Schnell, 2012). The mechanism is supposed to involve competitively binding of the inhibitor to α-glucosidase enzymes as opposed to carbohydrate thus interfering the synthesis of monosaccharides (E, 1997). Additionally, α-glucosidase inhibitors are also in use for biotechnological purposes and oligosaccharide synthesis of specific type (Tiengburanatam *et al*., 2010).

Haemolysis, red blood cells destruction causes many physiological and pathological problems (Dhaliwal *et al*., 2004) either *via* intravascular and extravascular hemolysis or due to gene malfunctioning (Bossu *et al*., 1968), the autoimmune problem (Gehrs and Friedberg, 2002) or external factors such as mechanical stress (Robinson *et al*., 2006), bacterial and viral infection (Kida *et al*., 1983, Huang *et al*., 1981). In nature, cholesterol and some sterols act as anti-hemolytic substances against several agents, while chemically, sterols resembling with natural bile acids showed distinct hemolytic properties (Berliner and Schoenheimer, 1938).

Bispyrazoles and bispyrazolones were reported as anti-inflammatory, antitumor, antimicrobial, antioxidant, anti-filarial, cytotoxic, anti-allergic, cardiovascular, diuretic, gastric secretion stimulatory and selective COX-2 inhibitory agents. These were also known to be good insecticides, herbicides, fungicides and industrially used in photography, paint, heat resistant polymer synthesis as well as for the analysis of urea in urine samples (Mehta *et al*., 2017, Abdul Wahab and Dawood 2012, Sharma *et al*., 2013, Ravindranathe *et al*., 2012, Hamama, 2001, Rosiere and Grossman, 1951, Bailey *et al*., 1985, Rao *et al*., 2014). A review of the literature revealed various

---

**Corresponding author:* e-mail: Chemiub14@gmail.com protocols have been adopted for condensation of aromatic/aliphatic/hetero-aromatic aldehydes with two equivalents of pyrazol-5-one using various catalyst such as piperidine, sodium lauryl sulphate (Metwally *et al*., 2012), ceric ammonium nitrate, cerium sulphate (Gupta *et al*., 2015), 2-hydroxyethyl ammonium acetate (Sobhani *et al*., 2012), melamine trisulfunic acid (Iravani *et al*., 2013), ionic liquid [HMIM]HSO_4 either under ultrasonic irradiation (Zang *et al*., 2001) or at reflux temperature (Zang *et al*., 2011). Similarly, recyclable catalysts such as ([Sipmim]HSO_4) (Baghernejad and Niknam, 2012), Zinc oxide nanowires (Eskandari *et al*., 2015), as well as condensation of phenylhydrazine, aromatic aldehydes with acetylene dicarboxylates (Gupta *et al*., 2015) or ethyl acetoacetate (Ravindranathe *et al*., 2012), are also reported. Diphenylic and mono phenylic bispyrazolones have been prepared under microwave irradiation or at reflux temperature (Huang *et al*., 1981) while catalyst-free synthesis has been carried out in polyethylene glycol-400 (PEG-400) at 110°C (Metwally *et al*., 2012). However, these approaches have disadvantages of using hazardous solvents, time-consuming, tedious workup, poor yields and nonfulfillment to green chemistry.

In continuation of our previous work (Ghafoor *et al*., 2016), we focused on the synthesis of bispyrazoles and their potential role as a hemolytic, antihemolytic and α-glucosidase agent.

MATERIALS AND METHODS

Chemicals, reagents, solvents were purchased from Merck (Germany) or Aldrich (USA) and used as such or purified where required by normal techniques. Reaction's progress was noted on Merck Silica gel 60 and F-254 precoated plates (0.25-mm thickness). Ultraviolet light (254 nm) was used to detect TLC Spots. Stanford Research Systems EZ-Melt automated melting point apparatus was used to observe melting points which were uncorrected. Proton and carbon NMR spectra were recorded on Agilent MR 400 NMR spectrometer operating at 1H frequency of 400MHz while Perkin Elmer RX1 FT-IR spectrometer was used to get IR Spectra. Mass spectra of high and low resolution were operated on a Thermo Finnigan LTQ FT instrument or Agilent TOF instrument and EIMS recorded on JEOL MS 600H-1.

General procedure for synthesis of 4-((5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl(aryl) methyl)-3-methyl-1-phenyl-1,2-dihydropyrazol-5-ones (4-14)

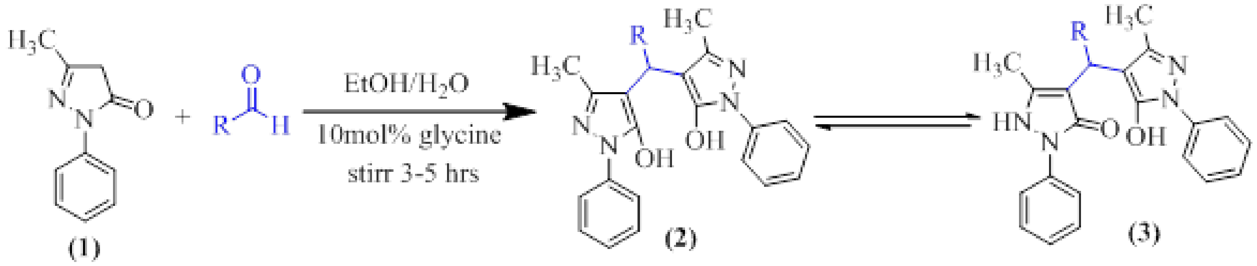
An aromatic aldehyde (1 mole) was dissolved in 15-20mL ethanol/water mixture (1:1) followed by addition of 2 mole of pyrazol-5-one (1). 10 mol % of glycine was added as catalyst and the mixture was stirred for 3-5 hours at room temperature. TLC was used for monitoring reaction finishing point. After completing reaction, crude product (2) or (3) was filtered, swept away with plenty of water and recrystallized with acetonitrile.

Compound No 4: White crystals, m.p = 183-184°C, Yield = 83%, IR: 1625 (C=O), 1582 (N=N), 1499, 1376, 1279/cm. ^{1}H-NMR (400 MHz, $(CD_3)_2SO$)): δ = 2.31 (2xCH_3, s, 6H), 5.11 (pyrazol-CH-thiophene, s, 1H), 6.73 (NH, s, 1H), 6.88-6.90 (thiophene-4-H, t, *J* = 4 Hz, 1H), 7.22-7.28 (thiophene-3,5-H, m, 2H), 7.41-7.45 (NH-N-PhH, t, *J* = 8 Hz, 5H), 7.68-7.70 (N=N-PhH, d, *J* = 8 Hz, 5H), 13.99 (pyrazol-5-OH, s, 1H). ^{13}C-NMR (400 MHz, $(CD_3)_2SO$)): δ = 11.94 (CH_3), 29.90 (pyrazol-C-thiophene), 121.03 (ArC), 124.51 (ArC), 124.58 (ArC), 126.09 (ArC), 127.19, 129.38 (pyrazol-N-C), 146.25 (C-OH), 147.94 (C=O). MS: *m/z* (%), 443 $[M^{+1}]^+$.

Compound No 5: White mass, m.p = 176-77°C, Yield = 79 %, IR: 1602 (C=O), 1579 (N=N), 1499, 1408, 1276, 1009/cm. ^{1}H-NMR (400 MHz, (CD_3CN): δ = 2.35 (2xCH_3, s, 6H), 5.02 (pyrazol-CH-furan, s, 1H), 6.18-6.19 (furan-5-H, d, *J* = 4 Hz, 1H), 6.34-6.36 (furan-3,4-H, t, *J* = 4 Hz, 2H), 7.26-7.29 (PhH, t, *J* = 8 Hz, 2H), 7.39 (2-NH, s, 1H), 7.43-7.47 (PhH, t, *J* = 4 Hz, 4H), 7.71-7.73 (PhH, d, *J* = 8 Hz, 4H), 13.42 (pyrazol-5-OH, s, 1H). ^{13}C-NMR (400 MHz, $(CD_3)_2SO$)): δ = 11.95 (CH_3), 28.71 (pyrazol-C-furan), 106.57, 110.81, 121.01 (ArC), 126.04 (ArC), 129.36 (ArC), 141.99 (pyrazol-N-C), 146.39 (C-OH), 154.56 (C=O). MS: *m/z*, 253 (20), 251.9 (100), 222.9, 173.9 (50), 153.9, 132, 118.9, 105, 90.9, 76.9.

Compound No 6: White powder, m.p = 152°C, Yield = 84 %, IR: 3071 (OH), 2920 (NH), 1597 (C=O), 1578 (N=N), 1497, 1412, 1370, 1286, 1162, 1024, 874/cm. ^{1}H-NMR (400 MHz, $(CD_3)_2SO$)): δ = 2.28 (2xCH_3, s, 6H), 4.74 (pyrazol-CH-furan, s, 1H), 6.33 (furan-2-H, s, 1H), 7.21-7.24 (furan-4,5-H, t, *J* = 4 Hz, 2H), 7.38-7.44 (PhH, q, *J* = 8 Hz, 6H), 7.51 (2-NH, s, 1H), 7.67-7.69 (PhH, d, *J* = 8 Hz, 4H), 13.96 (pyrazol-5-OH, s, 1H). ^{13}C-NMR (400 MHz, $(CD_3)_2SO$)): δ = 11.93 (CH_3), 25.48 (pyrazol-C-furan), 111.53, 121.03, 126.03 (ArC), 126.91 (ArC), 129.35 (ArC), 139.72 (pyrazol-N-C), 143.40 (C-OH), 145.99 (C=O). MS: *m/z*, 253 (47), 251.9 (100), 223, 195, 174 (88), 155, 132, 119, 105, 91, 77, 51.

Compound No 7: Yellow solid, m.p = 192-93°C, Yield = 86%, IR: 2985 (OH), 2922, 2877 (NH), 1591 (C=O), 1500, 1370, 1281, 1199, 1122, 1066/cm. ^{1}H-NMR (400 MHz, $(CD_3)_2SO$)): δ = 2.10 (2xCH_3, s, 6H), 4.16 (dioxane-2xCH_2, s, 4H), 4.70 (pyrazol-CH-Ph, s, 1H), 6.66-6.74 (C-PhH, q, *J* = 11 Hz, 3H), 7.21-7.25 (PhH, t, *J* = 8 Hz, 2H), 7.41-7.44 (PhH, t, *J* = 8 Hz, 4H), 7.68-7.70 (PhH, d, *J* = 8 Hz, 4H), 13.98 (pyrazol-5-OH, s, 1H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 11.46 (CH_3), 32.88 (pyrazol-C-Ph), 64.24 (dioxane-C), 64.32 (dioxane-C), 105.63, 116.04, 117.01, 120.04 (ArC) 121.33 (ArC), 126.17 (ArC), 128.83 (ArC), 134.10 (ArC), 136.90, 142.02 (Ar-C-OCH_2), 143.16 (pyrazol-N-C), 146.29 (C-OH), 157.51 (C=O). MS: *m/z*, 321 (78), 320 (100), 319 (99), 289, 263, 236, 214, 185, 174 (41), 149, 115, 91, 77, 51.



Scheme 1: Synthetic pathway of novel compounds 4-12.

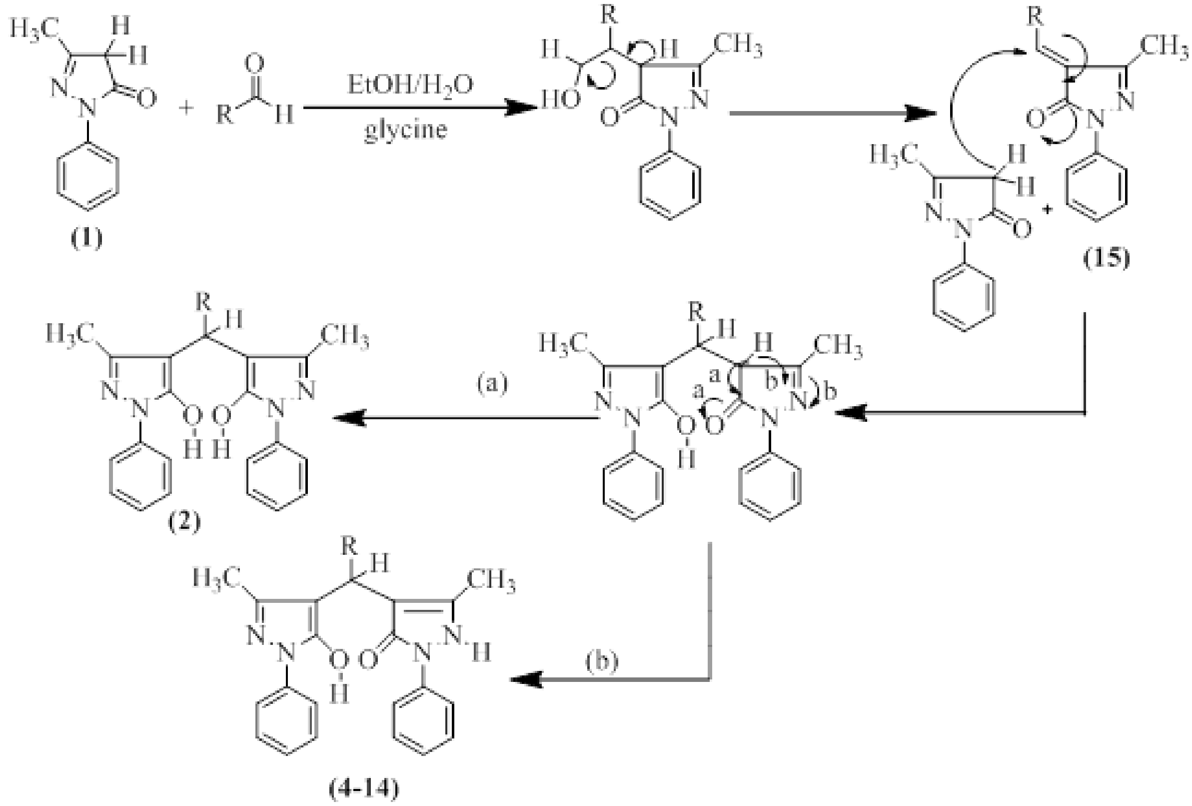


Fig. 1: Reaction mechanism.

Compound No 8: Light orange crystals, m.p = 147°C, Yield = 72 %, IR: 1598 (C=O), 1577, 1500, 1421, 1284, 1091/cm. ^{1}H-NMR (400 MHz, $(CD_3)_2SO$)): δ = 2.32 (2xCH_3, s, 6H), 5.0 (pyrazol-CH-indol, s, 1H), 6.32 (NH, s, 1H,), 6.97-7.00 (indol-H, dd, J_1 = 4 Hz, J_2 = 4Hz, 1H), 7.22-7.27 (indol-H, m, 4H), 7.39-7.44 (PhH, q, J = 7 Hz, 5H), 7.69-7.71 (PhH, d, J = 8 Hz, 5H), 10.92 (indol-NH, b, 1H). ^{13}C-NMR (400 MHz, $(CD_3)_2SO$)): δ = 19.01 (CH_3), 33.69 (pyrazol-C-indol), 56.49, 101.44, 111.48, 118.39 (ArC), 120.90 (ArC), 121.44 (ArC), 125.74 (ArC), 127.94 (ArC), 129.36 (ArC), 132.92 (pyrazol-N-C), 134.92 (C-OH), 146.69 (C=O). MS: *m/z*, 301 (100), 300 (100), 272, 259, 232, 185, 174 (60) 150.6, 130, 105, 91, 77, 51.

Compound No 9: White powder, m.p = 163-64°C, Yield = 84 %, IR: 3123 (OH), 1600 (C=O), 1578, 1501, 1381, 1270, 1179, 1058, 815/cm. ^{1}H-NMR (400 MHz, $(CD_3)_2SO$)): δ = 2.31 (2xCH_3, s, 6H,), 4.95 (pyrazol-CH-Ph, s, 1H), 7.14 (imidazol-4-H, s, 1H), 7.19-7.22 (C-Ph-2,6-H, t, J = 4 Hz, 2H), 7.37-7.43 (N-PhH, q, J = 8 Hz, 6H), 7.51-7.53 (C-Ph-3,5-H, d, J = 8 Hz, 2H), 7.69 (imidazol-5-H, s, 1H), 7.72-7.74 (N-PhH, d, J = 8 Hz, 4H), 8.27 (imidazol-2-H, b, 1H). ^{13}C-NMR (400 MHz, $(CD_3)_2SO$)): δ = 12.28 (CH_3), 33.40 (pyrazol-C-Ph), 104.53 (imidazol-C) 118.88, 120.79 (ArC), 120.97 (ArC), 125.71 (ArC), 129.02, 129.29 (ArC), 135.17, 136.15 (imidazol-C), 138.19 (pyrazol-N-C), 142.42 (C-OH), 146.64 (C=O). MS: *m/z*, 328 (14), 195, 185, 174 (100), 145, 132, 105, 91, 77, 64, 51, 41.

Compound No 10: White solid, m.p = 235-36°C, Yield = 84 %, IR: 2913 (NH), 1577 (C=O), 1497, 1414, 1292, 1029, 754/cm. ^{1}H-NMR (400 MHz, $(CD_3)_2SO$)): δ = 2.32 (2xCH_3, s, 6H), 5.03 (pyrazol-CH-pyridine, s, 1H), 7.21-7.25 (pyridin-5,6-H, t, J = 8 Hz, 2H), 7.31-7.34 (NH, q, J

= 4 Hz, 1H), 7.40-7.44 (PhH, t, *J* = 8 Hz, 5H), 7.68-7.70 (PhH, d, *J* = 8 Hz, 5H), 8.38-8.39 (pyridin-4-H, d, *J* = 4 Hz, 1H), 8.47 (pyridine-2-H, s, 1H). ^{13}C-NMR (400 MHz, $(CD_3)_2SO$)): δ = 12.11 (CH_3), 31.51 (pyrazol-C-pyridine), 104.23, 121.03 (CAr), 123.82 (CAr), 126.04 (CAr), 129.35 (CAr), 135.77, 137.78 (pyrazol-N-C), 138.48, 146.61 (pyridine-C) 147.16 (C-OH), 148.84 (C=O). MS: *m/z*, 264 (24), 263 (100), 234, 185, 174 (64), 145, 132, 105, 91, 77, 51.

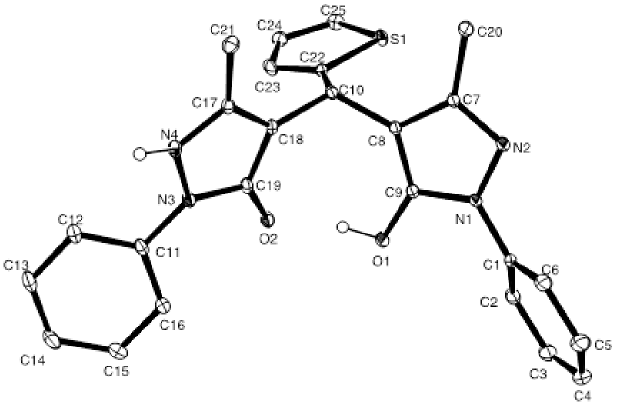


Fig. 2: X-ray Crystal of compound No 4

Compound No 11: Yellow powder, m.p = 158-59^{o}C, Yield = 79 %, IR: 3078 (OH), 1599 (C=O), 1522 (NO_2), 1503, 1492, 1347 (NO_2), 1268, 1181/cm. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.13 (2x$CH_{3,}$ s, 6H,), 4.83 (pyrazol-CH-Ph, s, 1H), 7.11-7.15 (C-Phenyl-2,6-H, t, *J* = 4 Hz, 2H), 7.28-7.32 (N-PhH, t, *J* = 8 Hz, 4H), 7.40-7.44 (C-Ph-3,5-H, t, *J* = 8 Hz, 2H), 7.58-7.60 (N-PhH, d, *J* = 8 Hz, 4H), 8.01-8.06 (N-PhH t, *J* = 7 Hz, 2H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 11.51 (CH_3), 33.47 (pyrazol-C-Ph), 104.81, 121.53, 122.23 (CAr), 126.55 (CAr), 128.95 (CAr), 129.29 (CAr), 133.69, 136.68, 143.03 (pyrazol-N-C), 146.22 (Ph-C-NO_2), 148.34 (C-OH), 157.45 (C=O). MS: *m/z*, 308 (7.7), 307 (37), 277, 185, 174 (100), 145, 132, 105, 91, 77, 57.

Compound No 12: Yellow solid, m.p = 163-64^{o}C, Yield = 86 %, IR: 1596 (C=O), 1479, 1403, 1297, 1199, 1071, 1013, 810, 747/cm. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.18 (2x$CH_{3,}$ s, 6H), 4.77 (pyrazol-CH-Ph, s, 1H), 7.09-7.10 (C-Ph-2,6-H, d, *J* = 4 Hz, 2H), 7.14-7.18 (C-Ph-3.5-H, t, *J* = 8 Hz, 2H), 7.31-7.37 (N-PhH, q, *J* = 8 Hz, 6H), 7.64-7.66 (N-PhH, d, *J* = 8 Hz, 4H). ^{13}C-NMR (400 MHz, $(CD_3)_2SO$): δ = 11.96 (CH_3), 33.04 (pyrazol-C-Ph), 119.47, 121.0 (CAr), 126.08 (CAr), 129.37 (CAr), 130, 131.37 (pyrazol-N-C), 142.05 (C-OH), 146.69 (C=O). MS: *m/z*, 343 (15), 342 (79), 341 (29), 340 (85), 261, 206.9, 185, 174 (75), 145, 128, 105, 91, 77, 51.

Compound No 13: White solid, m.p = 173^{o}C, Yield = 83 %, IR: 2232 (CN), 1595 (C=O), 1499, 1407, 1295, 1197, 1023, 910, 839, 816, 75/cm. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.13 (2x$CH_{3,}$ s, 6H,), 4.79 (pyrazol-4-CH-Ph, s, 1H), 7.14-7.17 (N-PhH, t, *J* = 6 Hz, 2H), 7.30-7.31 (C-Ph-2,6-H, d, *J* = 4 Hz, 2H), 7.32-7.35 (N-PhH, dd, J_1 = 4Hz, J_2 = 8 Hz, 4H), 7.52-7.54 (C-Ph-3,5-H, d, *J* = 8 Hz, 2H) 7.61-7.63 (N-PhH, d, *J* = 8 Hz, 4H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 13.18 (CH_3), 33.86 (pyrazol-C-Ph), 105.02, 110.17, 118.92 (CAr), 121.38 (CAr), 126.49 (CAr), 128.11, 128.98 (CAr), 129.88 (CAr), 132.15, 133.26 (CAr), 136.79, 146.32 (pyrazol-N-C), 146.41 (C-OH), 157.62 (C=O). MS: *m/z*, 289 (4), 288 (19), 287 (90), 258, 185, 174 (67), 154, 127, 105, 91, 77, 51.

Compound No 14: White solid, m.p = 166^{o}C, Yield = 89 %, IR: 3130 (OH), 1697 (C=O), 1594, 1507, 1314, 1113, 1000/cm. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.24 (2x$CH_{3,}$ s, 6H), 4.83 (pyrazol-CH-Ph, s, 1H), 7.16-7.23 (N-PhH, m, 2H), 7.33-7.37 (N-PhH, t, *J* = 8 Hz, 4H), 7.43-7.45 (C-Ph-2,6-H, d, *J* = 8 Hz, 2H), 7.65-7.67 (N-PhH, d, *J* = 8 Hz, 4H), 8.01-8.03 (C-Ph-3,5-H, d, *J* = 8 Hz, 2H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 11.75 (CH_3), 121.19, 126.23 (CAr), 128.43 (CAr), 128.62, 128.96 (CAr), 139.70, 146.40 (pyrazol-N-C), 151.11 (C-OH), 159.63 (C=O). MS: *m/z*, 471.42 [M+1], 472.42 [M+2], 473.42 [M+3] 297.33, 175.25.

In-vitro α-glucosidase, hemolytic and anti-hemolytic screening

These biological activities were performed in biochemistry lab, Department of Chemistry, The Islamia University of Bahawalpur. Reported procedures (Rahim *et al*., 2015, Yar *et al*., 2015) were adopted to study these properties

RESULTS

In the contemporary scheme of work, various aldehydes with two equivalents of (1) went through one-pot tandem Knoevenagel-Michael reaction to give the analogous bispyrazoles at room temperature (4-14) in 72-89% yields (Scheme 1). The synthesis of the final products (4-14) was confirmed by analytical techniques such as NMR, IR, Mass spectrometry and 4 by crystallography. Furthermore, these were tested for α-glucosidase, hemolytic and anti-hemolytic activities and showed moderate to good activities.

DISCUSSION

The synthesis of bispyrazoles is believed to involve an initial tandem reaction of aldehydes with (1) under the catalytic action of glycine to form the knoevenagel product (15), which underwent a Michael addition by another molecule of (1) to produce the bispyrazolone (Sobhani *et al*., 2012) shown in fig. 1.

The synthesis of the final products (4-14) were confirmed by different analytical techniques. In ^{1}H-NMR spectra of all products, six protons of methyl were represented by

Table 1: Synthesis of oxino bis-pyrazoles (4-14) from different aldehydes

Comp’d	R	Time (h)	Melting Point (˚C)	Yield (%)
4	Thien-2-yl	4	183-184	83
5	Furan-2-yl	3	176-177	79
6	Furan-3-yl	3.5	152	84
7	Benzo(*b*)(1,2)dioxanyl	4.5	192-193	86
8	5-Indolyl	4	147	72
9	Phenyl-4-(1*H*)imidazolyl	5	163-164	84
10	Pyridin-3-yl	4	235-236	84
11	4-Nitrophenyl	4.5	158-159	79
12	4-Bromophenyl	4	163-164	86
13	4-Cyanophenyl	4	173	83
14	4-Chlorophenyl	3	166	89

Table 2: Anti-α-glucosidase activities of the compounds 4-14

Comp. No	Anti-α- Glucosidase activity (0.5mM)	IC_{50} µM
4	72.23±0.21	286.56±0.16
5	65.56±0.25	351.24±0.19
6	92.28±0.14	37.48±0.11
7	91.42±0.17	89.25±0.13
8	92.74±0.15	32.34±0.12
9	62.53±0.23	396.25±0.18
10	89.26±0.19	152.42±0.15
11	92.23±0.16	31.26±0.11
12	88.75±0.21	154.87±0.16
13	91.26±0.17	65.23±0.12
Stand.	(Acarbose) 92.23±0.16	37.38±0.12

Table 3: Haemolysis and anti-haemolysis activities of the compounds 4-14

Comp. No	Average Haemolysis %±S.D (0.5mM)	IC_{50} µM	Average Anti-haemolysis %±S.D (0.5mM)	IC_{50} µM
4	53.7±0.99	227	11.3±1.2	-
5	45.67±2.14	-	13.3±1.8	-
6	32.2±1.8	-	13.9±1.3	-
7	53.3±2.34	393	18.1±3.3	-
8	61.43±1.15	296	24.2±1.96	-
9	48.2±0.38	-	32.1±1.3	-
10	19.93±1.3	-	19.6±0.8	-
11	57.3±1.3	317	34.2±0.36	-
12	31.3.3±1.8	-	15.7±1.1	-
13	34.93±4.01	-	53.77±0.63	458
Stand.	(Troiton) 98.76±0.92			-

Methanol was used to prepare solution. Triplicate measurements were done for all. Mean ± SEM was calculated for results.

one signal (singlet) in the range of δ = 2.10-2.35 and a specific singlet of Ar-CH at δ = 4.70-5.11. Aromatic hydrogens are observable in their respective region while hydroxyl signal is de-shielded and is observable only in (4-6) at δ = 13.42-13.99. In high and low-resolution mass spectra, the M+1 peak was observed for the compounds (4) and (14), but in EIMS, M+ peak was not observed rather an M-174 peak appeared in all the spectra in addition to other fragments (Sobhani *et al*., 2012). X-ray of bispyrazole (4), showed hydrogen attached to the second nitrogen of pyrazole ring (fig. 2) which is also in accord with the literature (Fun *et al*., 2009).

Reaction condition was fairly general and found to be independent of the nature of aldehydes and equally worked for substituted aldehydes, hetero-aromatic and condensed aromatic aldehydes. All reactions proceeded well to give products (4-14) in fairly good or excellent yields (table 1). The benefit of the current technique is its simplicity, simple work-up and formation of almost a pure product without the need for further purification.

The newly produced compounds (4-14) were designated for their α-glucosidase, hemolytic and anti-hemolytic activities (table 2). Haemolytic and anti-hemolytic activities were carried out by modified Malagoli's process (Malagoli, 2007) using positive control (Triton X-100 (0.1% v/v) and PBS as a negative control while α-glucosidase activity in yeast cell using acarbose as a standard (Chapdelaine *et al.*, 1978). Most of the compounds were found to be active agents but only α-glucosidase results were comparable to the standard (acarbose), where compound (8) and (11) were found even more active than the standard.

Result of α-glucosidase activity clearly indicates that few compound (6, 8 & 11) have IC_{50} value equal or below standard drug. Thus, these compounds can be further tested for cytotoxicity to use as potential drug. Structure analysis indicates that benzyl as aryl group having nitrogen bearing substituent shows better result than nitrogen being part of ring except 8. Similarly, furan-3-yl shows better result than furan-2-yl, which indicates position of heteroatom also effects the activity. All compounds showed haemolytic and anti-haemolytic activity but IC_{50} value was not promising.

CONCLUSION

In the current study, the synthesis of some new oxino bis-pyrazoles (4-14) are reported via environmental friendly way using cheap, eco-friendly and easy available glycine catalyst. Resultant compounds were analyzed by NMR, mass spectrometry, IR and X-ray crystallography (for compound 4) and tested for their hemolytic, anti-hemolytic and anti α-glucosidase activities. In future, these compounds can be tested for cytotoxicity to use as important pharmacological drugs. As far as, other biological activities such as antibacterial and antifungal are under consideration.

ACKNOWLEDGEMENT

Authors have no conflict for publishing this article and acknowledges the financial support by HEC in the form of Indigenous PhD scholarship & IRSIP. Primary author also acknowledges Bio-21 institute of University of Melbourne for providing support for some experimental work and characterization techniques. Acknowledgement is also due to, The Islamia University of Bahawalpur & The Government Sadiq College Women University, Bahawalpur for providing chemicals and performing biological activities.

REFERENCES

Baghernejad M and Niknam K (2012). Synthesis of 4,4’-(Arylmethylene)bis(1H-pyrazol-5-ols) Using Silica-bonded Ionic Liquid as Recyclable Catalyst. *Int. J. Chem.*, **4**: 52-60.

Bailey DM, Hansen PE, Hlavac AG, Baizman ER and Pearl J (1985). 3,4-Diphenyl-1H-pyrazole-1-propanamhe Antidepressants. *J. Med. Chem.*, **28**: 256-260.

Berliner F and Schoenheimer R (1938). Hemolytic and antihemolytic properties of bile acids and sterols in relation to their structures. *J. Biomed. Chem.*, **124**: 525-541.

Abdel-Wahab BF and Dawood KM (2012). Synthesis and applications of bipyrazole systems. *Arkivoc*, **1**: 491-545.

Bossu M, Dachà M and Fornaini G (1968). Neonatal hemolysis due to a transient severity of inherited pyruvate kinase deficiency. *Acta Haematolo.*, **40**: 166-175.

Chapdelaine P, Tremblay RR and Dube' JY (1978). P-Nitrophenol-alpha-D-glucopyranoside as substrate for measurement of maltase activity in human semen. *Clin. Chem.*, **24**: 208-311.

Dhaliwal G, Cornett PA and Tierney LM (2004). Hemolytic anemia. *Am. Fam. Physician.*, **69**: 2599-2606.

Lebovitz HE (1997). alpha-Glucosidase inhibitors. *Endocrinol Metab Clin North Am.*, **26**: 539-551.

Eskandari K, Karami B, Khodabakhshi S and Hoseini SJ (2015). Green and efficient synthesis of novel bispyrazole through a tandem Knoevenagel and Michael type reaction using nanowire zinc oxide as powerful and recyclable catalyst. *Turk. J. Chem.*, **39**: 1069-1077.

Fun HK, Kia R, Girish KS and Kalluraya B (2009). 4-[(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)phenylmethyl]-5-methyl-2-phenyl-1H-pyrazol-3(2H)-one ethanol hemisolvate. *Acta Crystallogr Sect E Struct Rep Online*, **65**: 066-067.

Gehrs BC and Friedberg RC (2002). Autoimmune hemolytic anemia. *Amm. J. Hematol.*, **69**: 258-271.

Ghafoor A, Aslam N, Elliott MC, Sajid N, Nasrullah M, Ashraf M, Ain Q and Khan MA (2016). Synthesis, characterization and biological evaluation of some new oxino bis-pyrazole derivatives. *Asian J. Chem.*, **28**: 1648-1652.

Gupta P, Gupta JK and Halve AK (2015). Synthesis and biological significance of pyrazolones: A review. *IJPSR.*, **6**: 2291-3010.

Hamama WS (2001). Pyrazolones as versatile precursors for the synthesis of fused and binary heterocycles. *Synth. Commun.*, **31**: 1335-1345.

Huang RT, Rott R and Klenk HD (1981). Influenza viruses cause hemolysis and fusion of cells. *Virology*, **110**: 243-247.

Iravani N, Albadi J, Momtazan H and Baghernejad M (2013). Melamine Trisulfunic Acid: An Efficient and Recyclable Solid Acid Catalyst for the Synthesis of 4,4′-(Arylmethylene)-bis-(1H-pyrazol-5-ols). *J. Chin. Chem. Soc.*, **60**: 418-424.

Kida H, Webster RG and Yanagawa R (1983). Inhibition of virus-induced hemolysis with monoclonal antibodies to different antigenic areas on the hemagglutinin molecule of A]seal]massaehuse-tts]l]80 (H7N7) Influenza virus. *Arch. Virol.,* **76**: 91-96.

Kim KY, Nguyen, TH, Kurihara H and Kim SM (2010). Alpha-glucosidase inhibitory activity of bromophenol purified from the red alga Polyopes lancifolia. *J. Food Sci.,* **75**: 145-150.

Malagoli D (2007). A full-length protocol to test hemolytic activity of palytoxin on human erythrocytes. *ISJ.,* **4**: 92-94.

Mehta HB, Patel PK, Dixit BC and Dixit RB (2017). Synthesis and antimicrobial activities of new mono and bisphenyl linked bispyrazole and bispyrazolone derivatives. *Arab. J. Chem.,* **10**: 1901-1912.

Metwally MA, Bondock SA, El-Desouky SE and Abdou MM (2012). Pyrazol-5-ones: Tautomerism, Synthesis and Reactions. *Int. J. Mod. Org. Chem.,* **1**: 19-54.

Rahim F, Ullah K, Ullah H, Wadood A, Taha M, Rehman AU, Uddin I, Ashraf M, Shaukat A, Rehman W, Hussain S and Khan KM (2015). Triazinoindole analogs as potent inhibitors of α-glucosidase: Synthesis, biological evaluation and molecular docking studies. *Bioorganic Chemistry,* **58**: 81-87.

Rao N, Prasad RG, Spoorthy YN, Rao R and Ravindranath LK (2014). *In vitro* microbiological evaluation of novel bis pyrazolones. *Ann. Pharm. Fr.,* **72**: 101-106.

Ravindranath LK, Kumar EVS, Srikant K, Spoorthi YN and Phebe P (2012). Synthesis of novel bis pyrazolones. *IJLPR.,* **2**: 144-149.

Robinson Y, Cristancho E and Böning D (2006). Intravascular hemolysis and mean red blood cell age in athletes. *Med. Sci. Sports Exerc.,* **38**: 480-483.

Rosiere CE and Grossman MI (1951). An Analog of Histamine that Stimulates Gastric Acid Secretion without other Actions of Histamine. *Science,* **113**: 651.

Sharma V, Khurana J M and Muralidhar K (2013). Spectrophotometric determination of urea in urine samples by using bispyrazolone method. *Proc. Indian Natl. Sci. Acad B. Biol. Sci.,* **79**: 51-56.

Sobhani S, Nasseri R and Honarmand M (2012). 2-Hydroxyethylammonium acetate as a reusable and cost-effective ionic liquid for the efficient synthesis of bis(pyrazolyl)methanes and 2pyrazolyl-1-nitroalkanes. *Can. J. Chem.,* **90**: 798-804.

Standl E and Schnell O (2012). Alpha-glucosidase inhibitors 2012- cardiovascular considerations and trial evaluation. *Diab. Vasc. Dis. Res.,* **9**: 163-169.

Stumvoll M, Goldstein BJ and Haeften TWV (2005). Type 2 diabetes: Principles of pathogenesis and therapy. *Lancet.,* **365**: 1333-1346.

Tiengburanatam N, Boonmee A, Sangvanich P and Karnchanatat A (2010). A novel α-glucosidase inhibitor protein from the rhizomes of *Zingiber ottensii* valeton. *Appl. Biochem. Biotechnol.,* **162**: 1938-1951.

Yar M, Bajda M, Shahzad S, Ullah N, Gilani MA, Ashraf M, Rauf A and Shaukat A (2015). Organocatalyzed solvent free an efficient novel synthesis of 2,4,5-trisubstituted imidazoles for α-glucosidase inhibition to treat diabetes. *Bioorganic Chemistry,* **58**: 65-71.

Zang H, Su Q, Guo S, Mo Y and Chang B (2011). An efficient one pot synthesis of pyrazolone derivatives promoted by acidic ionic liquid. *Chin. J. Chem.,* **29**: 2202-2204.

Zang V, Su Q, Mo Y and Cheng B (2001). Ionic liquid under ultrasonic irradiation towards a facile synthesis of pyrazolone derivatives. *Ultrason. Sonochem.,* **18**: 68-72.